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THE SURVIVAL OF HUMAN ENTERIC VIRUSES IN HOLDING PONDS.(U)
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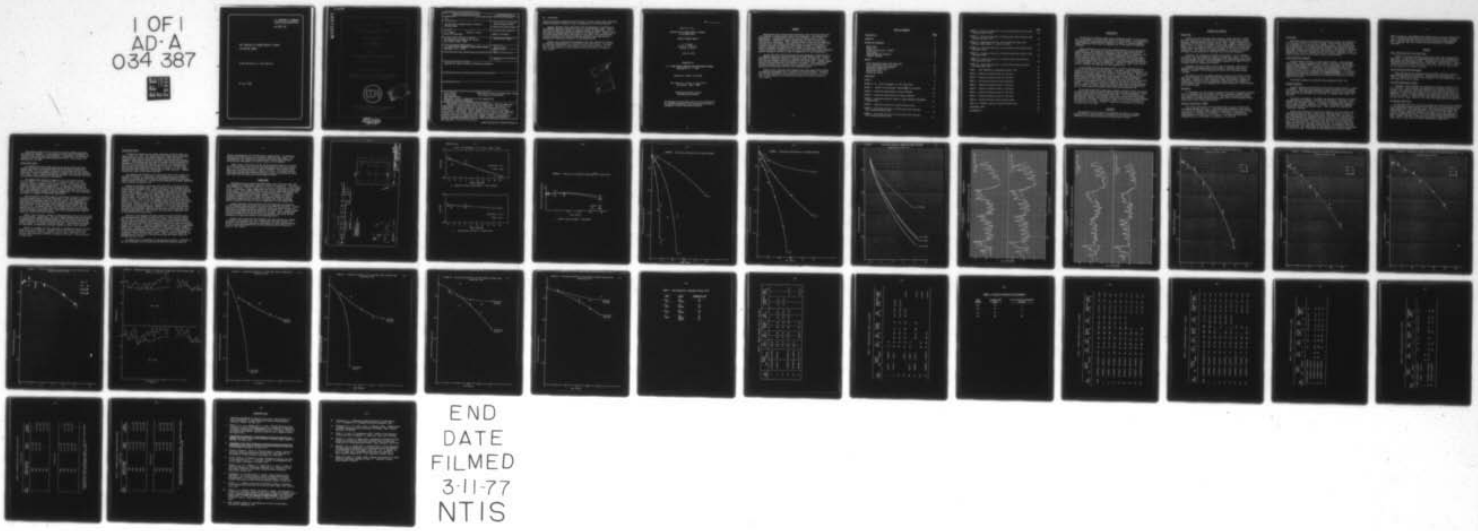

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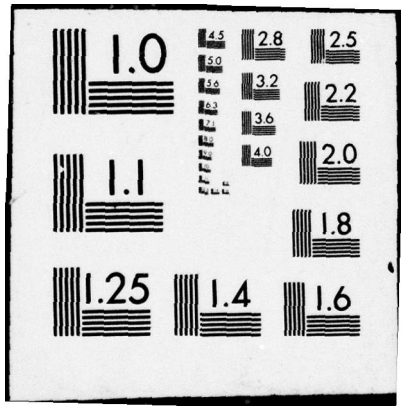
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THE SURVIVAL OF HUMAN ENTERIC VIRUSES IN HOLDING PONDS

TEXAS UNIVERSITY AT SAN ANTONIO

19 JULY 1976

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Report No. 76-1

The Survival of Human Enteric Viruses in Holding Ponds

Annual Progress Report

B. P. Sagik
Steven W. Funderburg
Barbara E. Moore

July 19, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DAMD 17-75-C-5062

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The University of Texas at San Antonio
San Antonio, Texas 78285

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Experimental data obtained from field models since 1975 have shown that virus removal in holding ponds involves two components: inactivation and sedimentation. Several factors may be cited as contributing to viral inactivation. Undoubtedly, higher temperatures can lead to a more rapid inactivation of poliovirus. Such a primary effect is seen in both laboratory controls and in the differences between winter and spring field results. A secondary influence of temperature and sunlight, however, is monitored in terms of increased biological activity. While the cumulative effects of

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Another important field observation was the appearance of poliovirus in the sediments generated during the life of the model holding ponds. Initial detection of infectious viruses in sediments during the winter run led to a more detailed sediment monitoring system for the spring tests. Results showed poliovirus being deposited at the bottom of the ponds. More significantly, virions continued to be recovered from the sediments long after the disappearance of infectious viruses in the overlying waters.

Similar viral recoveries from sediments have been reported for viruses in estuarine environments and in laboratory pond water systems. Therefore, what has been reported in some studies as viral inactivation may, in fact, have included deposition of particulate-associated virions as a means of viral removal.

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SUMMARY

Experimental data obtained from field models since 1975 have shown that virus removal in holding ponds involves two components: inactivation and sedimentation. Several factors may be cited as contributing to viral inactivation. Undoubtedly, higher temperatures can lead to a more rapid inactivation of poliovirus. Such a primary effect is seen in both laboratory controls and in the differences between winter and spring field results. A secondary influence of temperature and sunlight, however, is monitored in terms of increased biological activity. While the cumulative effects of various microbiotic communities are difficult to dissect under field conditions, general biological activity is seen as antagonistic to viral populations.

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INTRODUCTION

The US Army is presently using several different methods of land application of wastewater at installations within the United States. Land application of wastewater is being given more emphasis as a means of ultimate disposal. Recent legislation will result in expanded use of this method of wastewater treatment.

Spray irrigation is one of the most popular methods of land application of wastewaters. The design and operational problems associated with this method of wastewater treatment are dependent upon a number of variables. The general topic has been covered in several comprehensive discussions (1-6). A major research need in the area of spray irrigation involves determination of the potential human risk of viral disease as a result of spraying sewage. It has been reported that the probability of inhaling pathogenic aerosols near a spray irrigation site may be significant. Results of recent field studies serve to validate the importance of this problem (7-8).

In the design of spray irrigation sites, a series of holding ponds is one of the unit processes often included. Such ponds, unlike conventional oxidation ponds, have a continuous influent, while withdrawal is limited to the growing or irrigation season. The latter is a regional variable. Thus, these ponds serve as holding impoundments accumulating in volume throughout the winter months and being drawn down almost entirely in the summer months. Detention periods are variable, but obviously can range from that time related to maximal pond capacity to almost no detention near the end of the spray season. In his review of the effectiveness of wastewater treatment processes for the removal of viruses, Sproul has indicated that there are insufficient data on virus removal by oxidation ponds under field conditions (9).

It has been shown that conventional secondary treatment cannot be expected to reduce virus concentrations by much more than one order of magnitude. This includes disinfection as currently practiced (10). The holding pond is the next and final point in the treatment chain where viruses can be inactivated. Unfortunately, specific survival data on human enteric viruses in ponds designed for this use are lacking. Consequently, there is a need for information on the effects of time, depth, sunlight, temperature and antagonistic organisms in these ponds on viruses.

OBJECTIVE

The objective of this study is to determine the effects of various holding periods, several depths of wastewater and natural environmental conditions on the long-term survival of human enteric viruses.

METHODS AND MATERIALS

Model Ponds

A series of twelve concrete tanks ranging in depth from four to eight feet were installed at the field site at the Center for Research in Water Resources, Balcones Research Center, Austin, Texas. Figure 1 indicates the excavation of the field and placement of the tanks (completed during July and August, 1975). In order to achieve a range of pond depths, six of the nine 4-foot tanks were backfilled with sand to depths of two feet and three feet, respectively. This results in operational depths of 18 inches, 30 inches, 42 inches, and 90 inches with a constant four to six-inch freeboard.

Initially, all ponds were lined with 6 mil plastic. As it developed the plastic liner was cumbersome, awkward to handle and leaked. At the end of the trial run, the plastic was removed from the ponds and they were sealed with Thoroseal® (dry mix). The Thoroseal® is prepared with ACRYL-60 and is routinely used as a swimming pool sealant.

Throughout the early months of the study considerable difficulty was experienced in eliminating leaks from the ponds. Practical success was achieved, although at this writing one of the 90-inch deep ponds remains a problem.

The shallower ponds are sampled within one inch of the surface and one inch from the bottom. An additional mid-depth sampling point is used in the deep ponds. Samples for viral, bacterial, algal, and chemical analyses are collected at the field site and immediately transported to the laboratory. Field readings taken at each sampling time include temperature, light intensity and dissolved oxygen.

Wastewater

All wastewater for this study is obtained from Govalle Wastewater Treatment Plant, a component part of the Austin municipal wastewater treatment system, and trucked to the model pond field site. The Govalle Wastewater Treatment Plant employs the contact-stabilization process with a 20 to 30-minute contact period and a 4-hour stabilization period.

Growth of Poliovirus I (CHAT)

The viral stocks used to seed the model ponds are grown in HeLa cells (calf serum adapted, Flow Lab.). Subconfluent HeLa monolayers are infected at a MOI of 10. Maximal viral harvests are obtained by three cycles of rapid freeze-thaw at 8-10 hours post infection. Cell debris is sedimented by centrifugation at 12,000 x g for 15 minutes. The resulting viral stocks, average titer 1-3 x 10^9 pfu/ml, are stored at 4 C until use.

Virus Assay

Poliovirus assays are performed by inoculating the HeLa cell monolayers on 60 mm plates with 0.2 or 0.3 ml of sample. Virus inocula are adsorbed for 45 minutes at 37 C with periodic rocking of plates. The infected cells are overlayed with 4 ml of Eagle's medium (modified) containing one percent Bacto-agar (Difco), 10 percent bovine serum (Flow), and antibiotics including penicillin, streptomycin, gentamicin, and mycostatin or fungizone. After incubation at 37 C in 2.5 percent CO_2 for 2 or 3 days, monolayers are stained using 2 percent neutral red in Hanks' Balanced Salt Solution.

Chemical/Physical Analyses

Routine chemical analyses on field samples include: total suspended solids (TSS), volatile suspended solids (VSS), total organic carbon (TOC), chemical oxygen demand (COD), nitrite- and nitrate-nitrogen, pH, dissolved oxygen and orthophosphate. (Originally, total phosphorous analyses were conducted but by mid-year it was replaced by orthophosphate.) All analytical procedures are in accordance with Standard Methods (11). In addition, temperature is monitored continuously at several pond depths. For the run beginning March 1976 light intensities are observed by photocell at various depths in the ponds.

Chlorophyll analyses are performed spectrophotometrically (12).

Sediment Sampling

Sediment samplers were made from cut-off plastic bleach bottles, 6 inches in diameter. Each bottle was weighted with about one-fourth inch of washed gravel and was then suspended from three lengths of nylon cord. After virus was seeded and the pond mixed for 15 minutes, 12 samplers were lowered into each pond.

The weekly accumulation of sediment was measured by removing one sampler and emptying out of sediment. The bottle was replaced in the pond. This sediment was designated "weekly". The amount of sediment which accumulated over a number of weeks, beginning at two weeks, was measured by sampling undisturbed bottles from separate ponds. This allowed the collection of "cumulative" sediment.

Samples were collected by raising the sampler to the pond surface and carefully decanting all but 2 liters of the overlying water. One liter of the remaining water was decanted and saved. The final liter was mixed to bring the sediment into suspension and then poured into a collection bottle. The sampler was then washed with the first liter and this too was poured into a collection bottle. The bottles were held at 4 C for 24 hours after which time the bulk of the water was poured off. The remaining liquid was mixed and poured into centrifuge bottles and spun at low speed (1,500 x G) for 10 minutes. The supernatent was aspirated off and the pellet resuspended in 200 ml of 1X PBS. Viral assay of the sediment was by direct plating.

After inoculation the sediments were removed from the cells by washing with 5 ml of 1X PBS containing 500 μ/ml of penicillin and 41.5 μ/ml of streptomycin. The wash was left on the cells for 15 minutes and then aspirated off. Monolayers then were overlaid with agar-base media as described previously.

RESULTS

Viral Interaction With 6 Mil Pond Liner

Volumes of secondary effluent and deionized water were inoculated with poliovirus. A strip of 6 mil transparent plastic liner was introduced into each volume and continually stirred. (Surface area to volume ratios were determined using the dimensions of a 42-inch pond.) Results shown in Figure 2 indicate that neither viral attachment to the liner nor viral inactivation in the presence of the plastic occurred.

Viral Interaction With Pond Sealer

After the plastic was replaced with Thoroseal®, evaluations similar to those described above were initiated. Results of this study shown in Figure 3 indicate that neither viral attachment nor viral inactivation occurs as a result of using the sealer.

Controlled Temperature Studies

Wastewater (final effluent) was seeded with poliovirus and held at 4 C, 20 C, and 30 C. Virus survival was determined as a function of time up to 50 days. The results of this study indicate a high survival rate at 4 C as compared to a relatively low survival rate at 20 C and 30 C (see Figure 3). Similarly, volumes of primary effluent were seeded with poliovirus and held at 4 C, 20 C and 30 C. Virus survival was significantly improved in primary effluent than in final effluent at all three test temperatures (see Figure 5).

Preliminary Field Tests

In September 1975, four of the model ponds were filled with final effluent. A seven-day period elapsed between the introduction of the wastewater and the viral inoculation of the shallower ponds. This interim period allowed the establishment of a large algal population in three of the four ponds. Conversely, no algal growth was visible in the deeper pond at the time this pond was seeded with poliovirus. This test period lasted approximately 30 days. Figure 6 and Table 1 represent data obtained during the preliminary field test for viral survival and mean temperature, respectively.

The primary purpose of this run was to evaluate sampling procedures and monitoring equipment. It was during this test that the inadequacy of the plastic as a pond liner was determined. Consequently, the ponds were drained, dried and the new sealer applied and cured. This process took approximately six weeks.

Winter Test Series

An expanded field test using eight of the ponds filled with final effluent was begun in mid-January and reflects the winter conditions of Central Texas. All four depths of pond were used during this test series. Ponds were filled with secondary effluent in January 1976 and were observed for 45 days. Viral, chemical and physical analyses (as previously described) were conducted during the entire test period.

The temperatures observed in ponds A-3, B-3, C-3 and D-3 can be found in Figure 7. It can be seen that in the shallow pond little difference is observed between the top and the bottom: both points reflect immediate changes in ambient temperatures. In contrast, differences as great as 9 C were observed between the top and the bottom in the 90-inch pond. The temperature in the bottom of this pond fluctuated little and did not respond to diurnal changes in ambient temperatures. It took an extended cold period to change the temperature appreciably.

Virus survival in the model ponds is illustrated in Figures 8 through 11. The ponds showed little or no real difference in virus survival between the bottom and top wastewater sample for the test period. The appearance of algae during the latter half of this period seems to have accelerated the viral decay rate in the shallower ponds (compare die-off between days 0-7 and days 7-25). In marked contrast, the decreased decay rate in the deep ponds during the first 25 days resulted in recovery of more than 10 percent of input virions as infectious virus. Only small differences were observed among top, middle and bottom fractions.

Tables 2 and 3 contain a summary of chemical/physical data for the 30-inch and 90-inch ponds, respectively. The major changes observed were the increase in percentage of volatile suspended solids in the top of both ponds and the retarded increase in volatile suspended solids in the bottom of the 90-inch pond as opposed to the bottom of the 30-inch pond. This reflects the growth of algae which, as previously indicated, appears to affect virus survival.

Table 4 is a summary of viral isolation in sediments of each of the test ponds at the end of this run. Although virus in the overlying waters was below the practical limits of detection after 31 days, there were 41 pfu isolated from each gram of sediment in pond B-3 at 67 days. This observation is significant.

Spring Test Series

A field test reflecting the spring conditions of Central Texas was begun on March 31, 1976. One shallow pond (30 inches) and one deep pond (90 inches) were filled with primary wastewater, while four ponds ranging in depth from 18 inches to 90 inches, were filled with secondary effluent. The following day each pond was inoculated with poliovirus to approximately 10^4 pfu/ml and mixed for 15 minutes. At the initiation of the run, no residual chlorine was detected in the final effluent ponds. Water samples were removed for viral assay at 1 hour, 1, 3, and 6 days and then once every week until viruses were no longer detected. Sediment samples were taken as previously described.

The temperatures recorded in B-3 (30 inches) and D-3 (90 inches) are presented in Figure 12. Once again, bottom temperatures are relatively constant in the deep pond while surface temperatures exhibited a more pronounced diurnal fluctuation. Average spring temperatures exceeded comparable winter values by as much as 10 C.

Figures 13 through 16 illustrate viral survival in the water of the 30-inch and 90-inch model ponds. (Since there is little discernible difference between survival at the various pond depths, only the data from the lowest level is given.) It is readily apparent that poliovirus is detected in the primary effluent ponds for a much longer time than in the final effluent ponds. Since temperature effects were nearly the same in both types of ponds, the reason for this difference lies in the nature of the effluent itself. Since it has been suggested that solids have protective effects on viruses (13, 14, 15), the greater amount of solids in the primary effluent may account, in part, for the higher virus levels maintained in these ponds. Dissolved oxygen, pH, chlorophyll levels and other chemical measurements of biological activity (Tables 5, 6, 7, and 8) indicate that the ponds containing final effluent were much more active than those containing primary effluent. This activity also may have an important role in the inactivation of poliovirus.

Analysis of the sediment from both primary and final effluent ponds revealed appreciable amounts of infectious virus. These results indicate that the loss of virions from the water is not due to inactivation alone. Results from weekly sediment samples presented in Tables 9 and 10 show that viruses began to settle with the sediment during the first week and continued through the fifth week. Cumulative sediment samples reveal that a significant number of the virions survived throughout the test period in the sediments. Additionally, extended poliovirus survival is observed in primary as opposed to final effluent sediments (Figures 13 through 16). The solids appear to concentrate viruses from the water column. These virions are deposited via sedimentation at the bottom of the pond where they may remain viable for extended periods of time.

Six weeks after the initiation of the spring test series, 33 percent of the test wastewater in the 30-inch and 90-inch final effluent ponds was

removed (approximately 107 and 400 gallons, respectively). No infectious poliovirus had been detected in the water columns for three weeks. The following day these volumes were replaced with "fresh" final effluent, and poliovirus was seeded at a level of about 3.0×10^4 pfu/ml.

After 3 days, virus levels in the 30-inch pond were down to 14 percent of the initial level, and at 8 days this had dropped to less than 0.01 percent. In the 90-inch pond after 3 days 57 percent of the virus was still detectable, and this was down to 5 percent in 8 days. In neither pond are these rates significantly different from the virus levels detected in these same ponds when the spring test series began in March.

CONCLUSIONS

Experimental data obtained from field models since 1975 have shown that virus removal in holding ponds appears to involve two components: inactivation and sedimentation. Several factors may be cited as contributing to viral inactivation. Undoubtedly, higher temperatures can lead to a more rapid inactivation of poliovirus. Such a primary effect is seen in both laboratory controls and in the differences between winter and spring field results. A secondary influence of temperature and sunlight, however, is monitored in terms of increased biological activity. While the cumulative effects of various microbiotic communities are difficult to dissect under field conditions, general biological activity is seen as antagonistic to viral populations.

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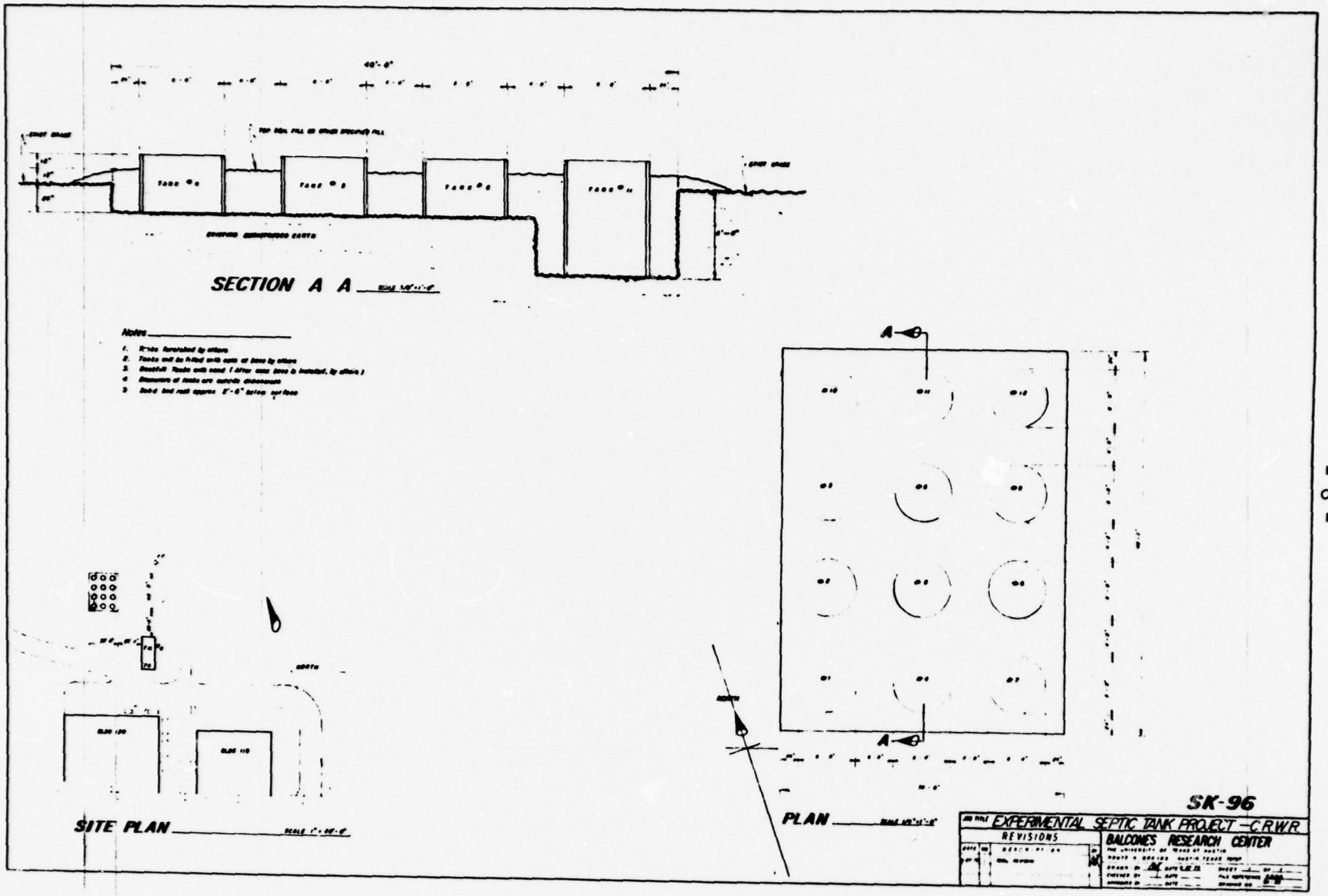
Similar viral recoveries from sediments have been reported for viruses in estuarine environments (16) and in laboratory pond water systems (17). Therefore, what has been reported in some studies as viral inactivation may, in fact, have included deposition of particulate-associated virions as a means of viral removal.

FIGURE 1.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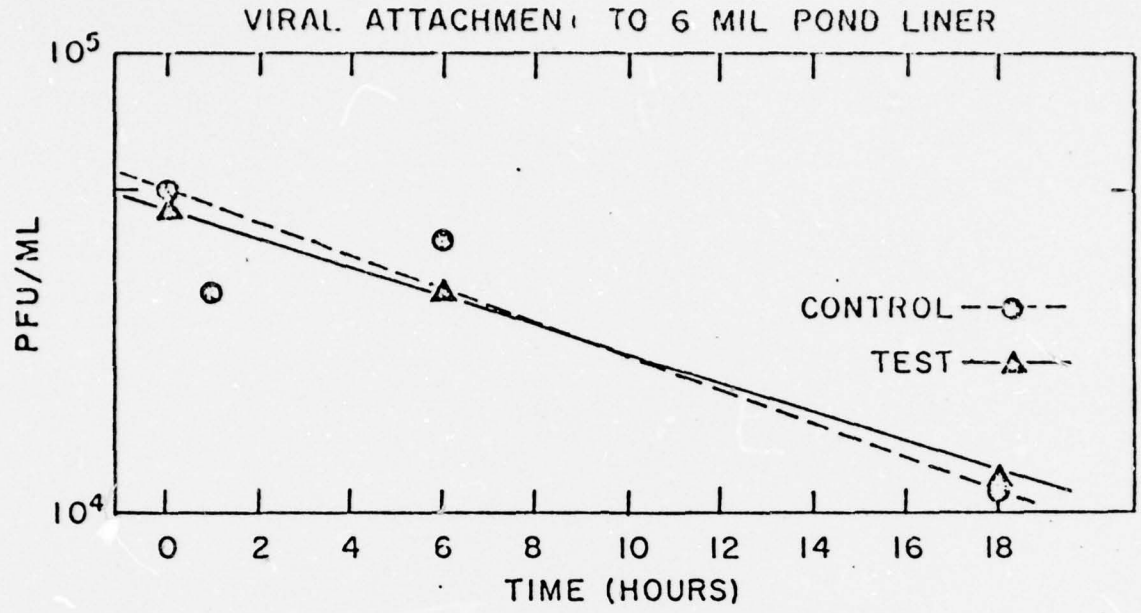
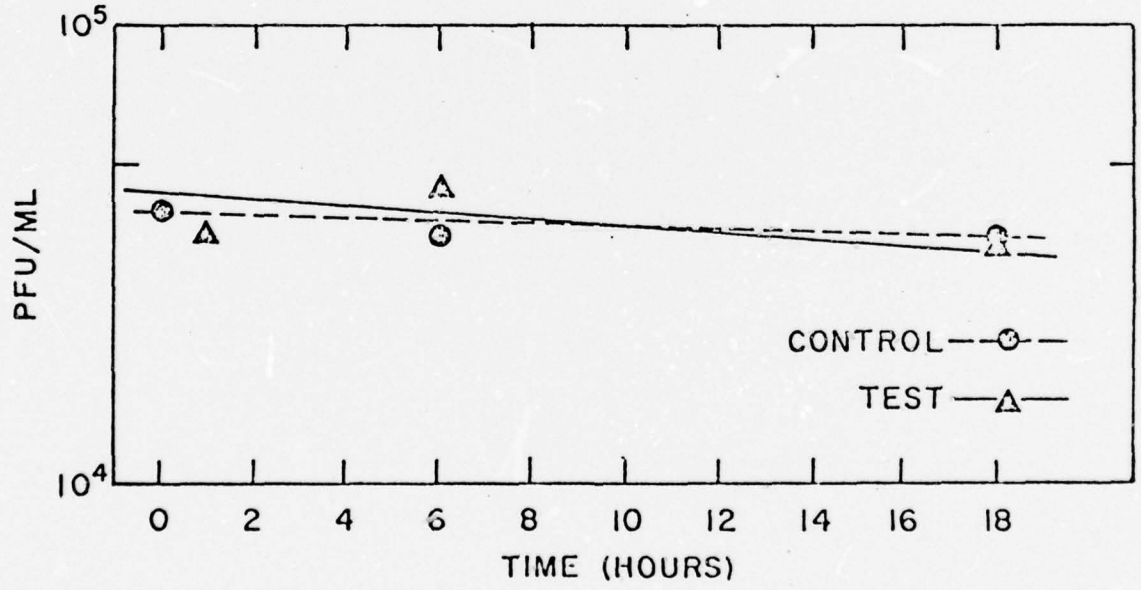
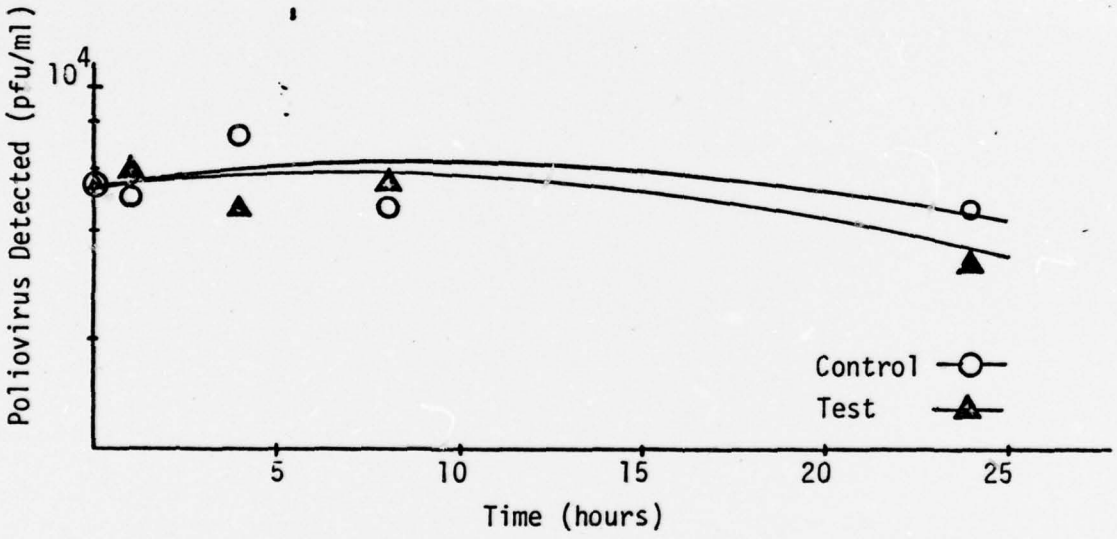


a. GOVALLE FINAL EFFLUENT + POLIOVIRUS



b. DEIONIZED WATER + POLIOVIRUS

FIGURE 3. Effect of Pond Sealant (Thoroseal®) on Poliovirus



c. GOVALLE FINAL EFFLUENT + POLIOVIRUS

FIGURE 4. Poliovirus Inactivation in Final Effluent

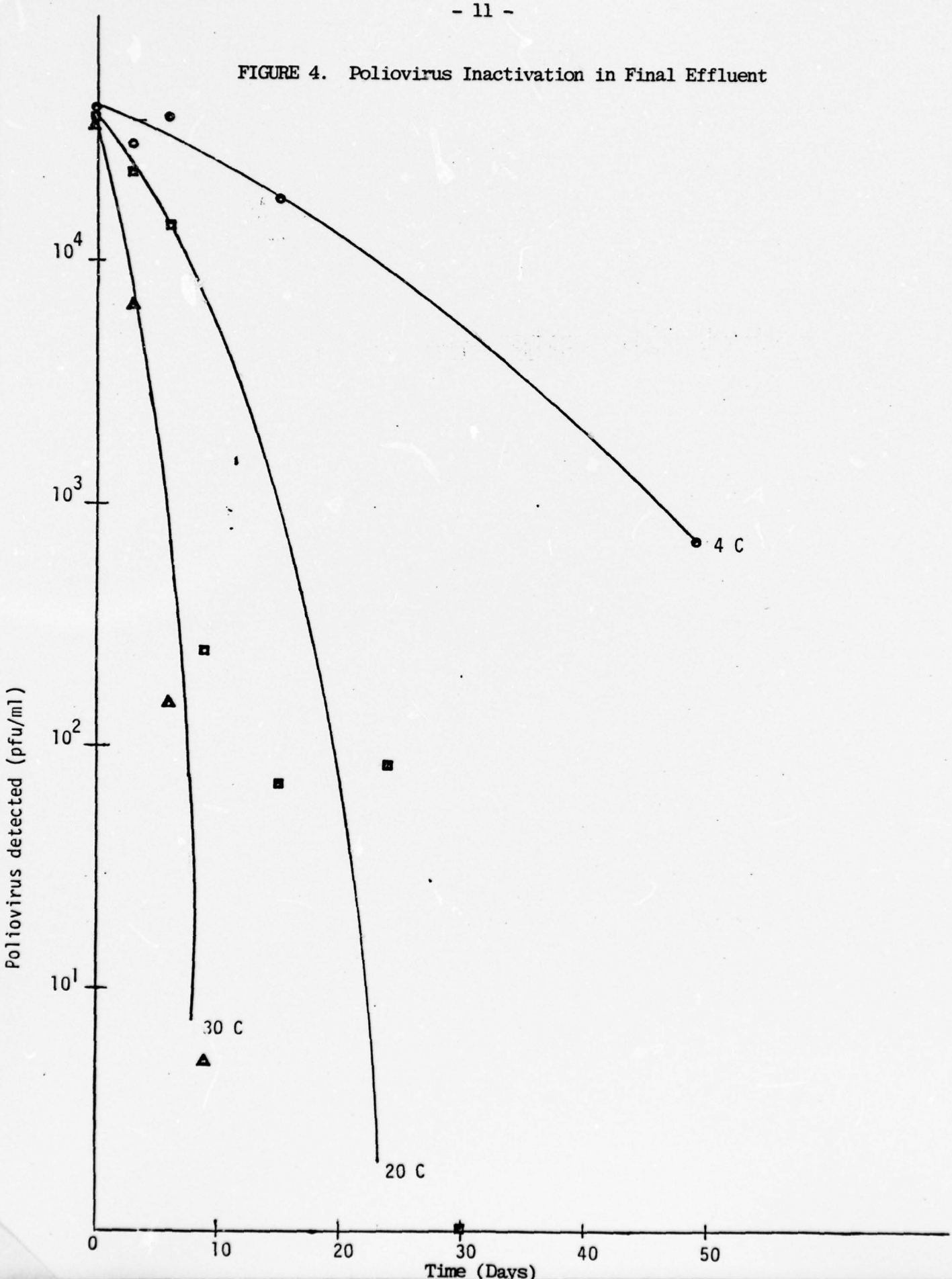


FIGURE 5. Poliovirus Inactivation in Primary Effluent

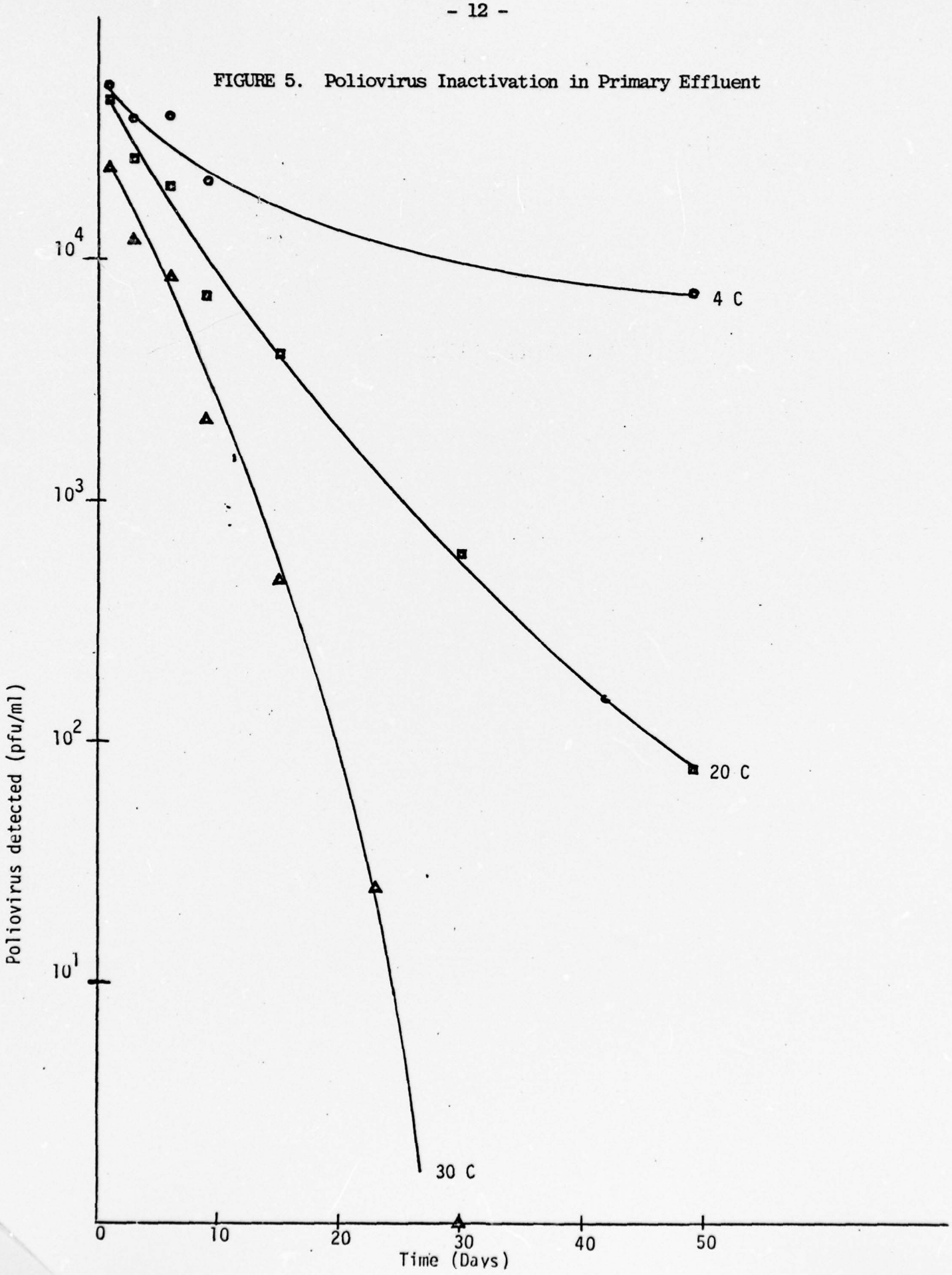


FIGURE 6. POLIOVIRUS SURVIVAL TRENDS IN FINAL EFFLUENT
(September-October 1975)

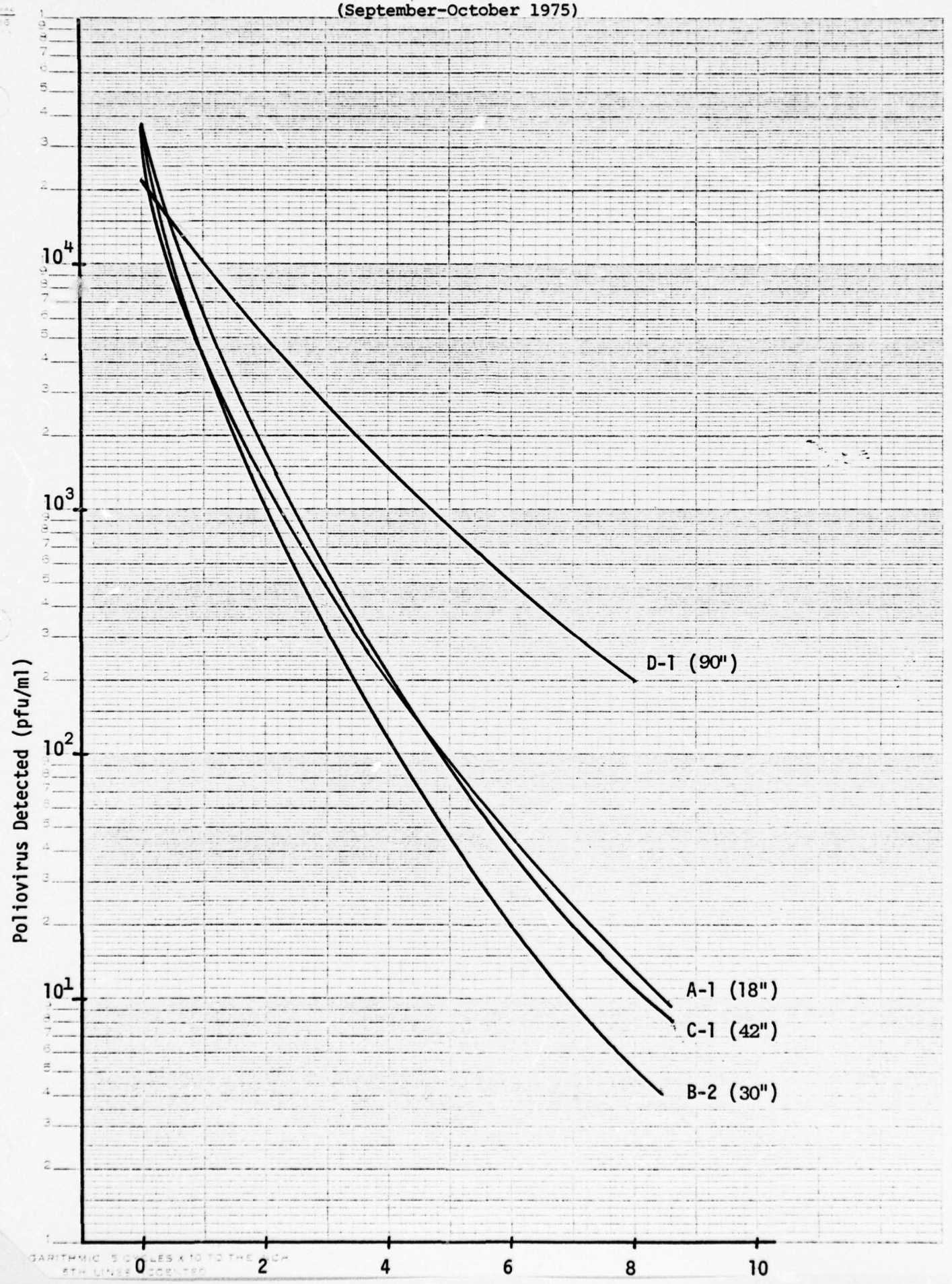


FIGURE 7. Temperature Profiles (January-February 1976)

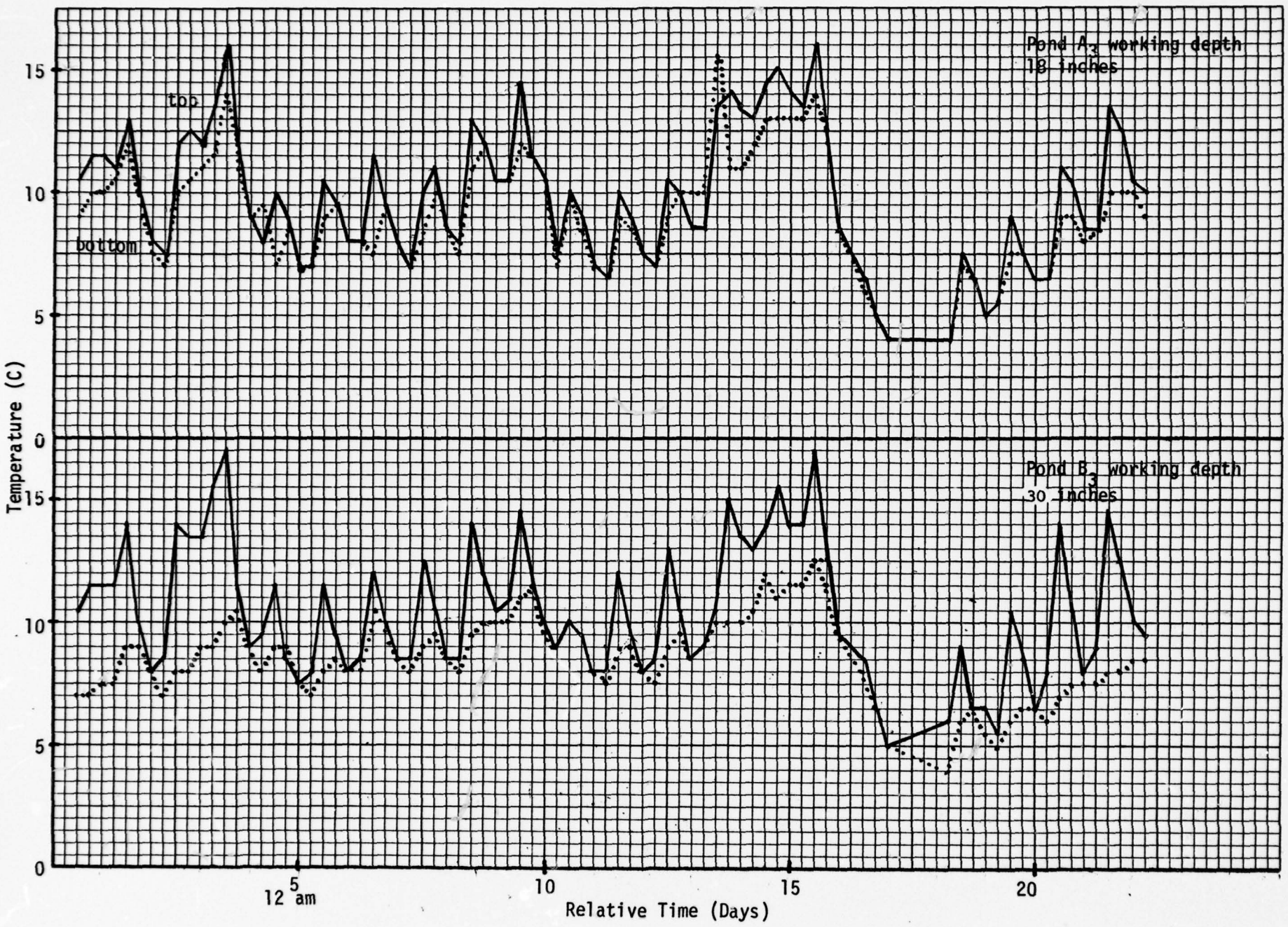


FIGURE 7 (continued). Temperature Profiles (January-February 1976)

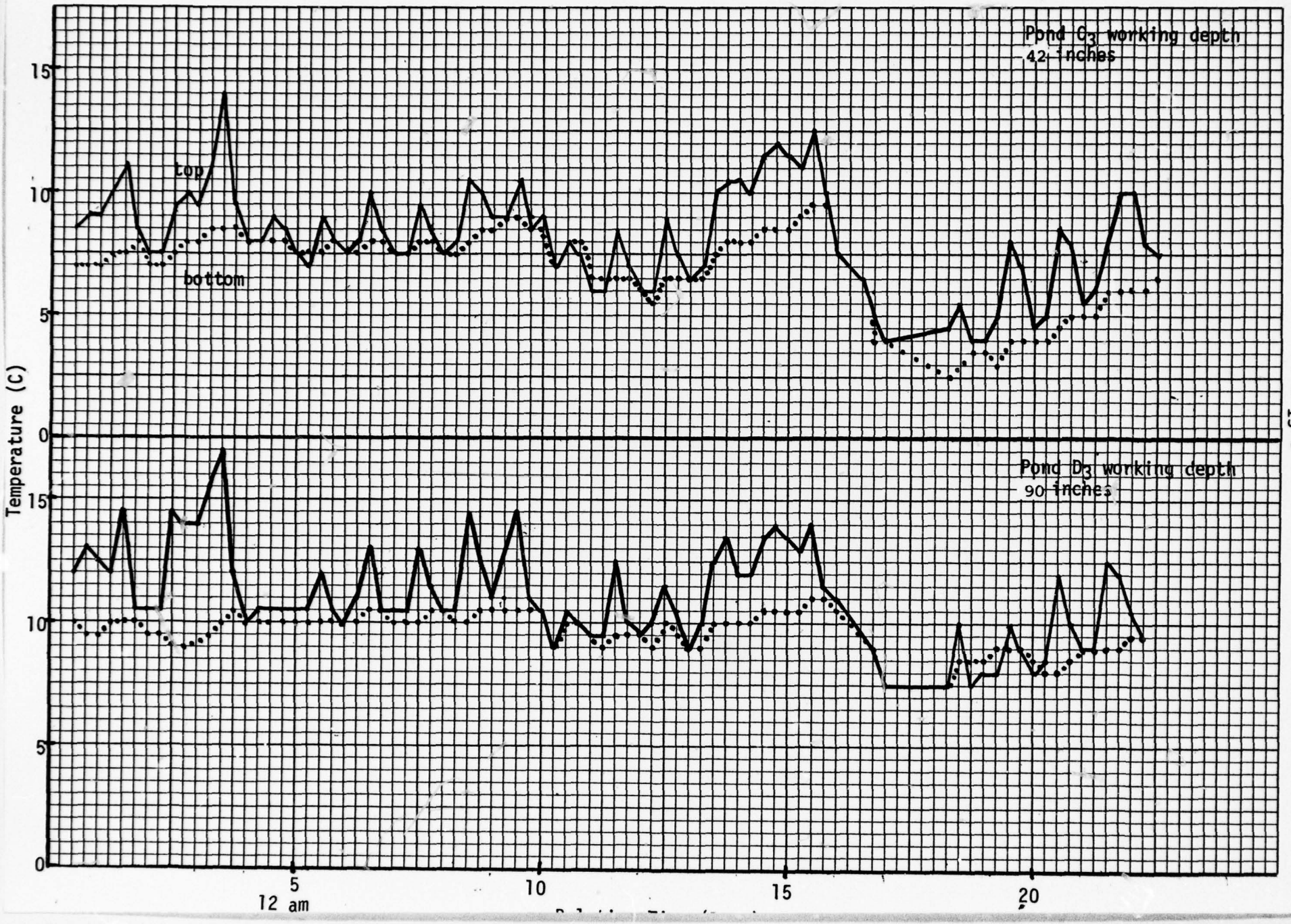


FIGURE 8. POLIOVIRUS SURVIVAL IN 18-INCH DEEP FINAL EFFLUENT POND
(January-February 1976)

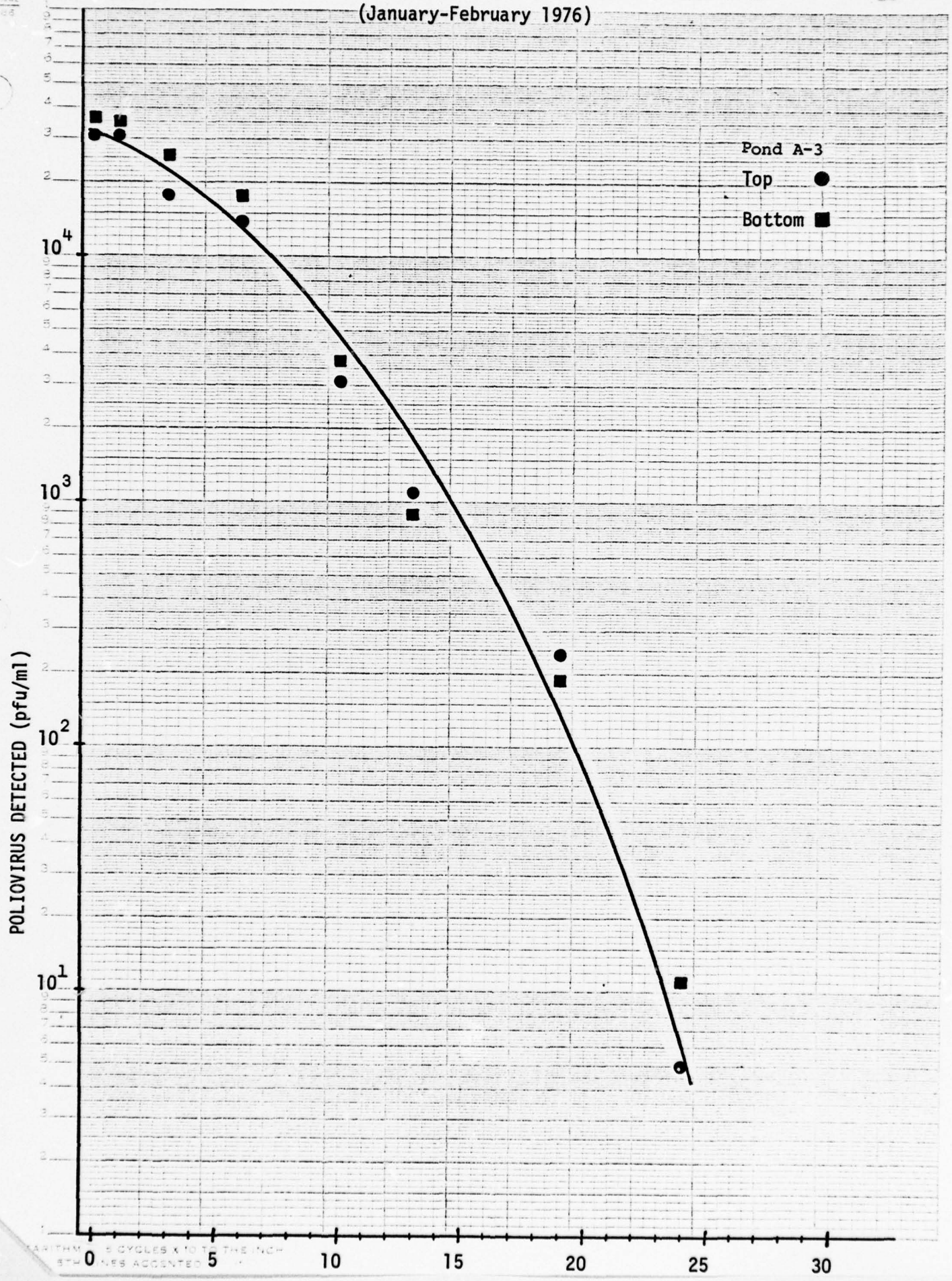


FIGURE 9. POLIOVIRUS SURVIVAL IN 30-INCH DEEP FINAL EFFLUENT POND
(January-February 1976)

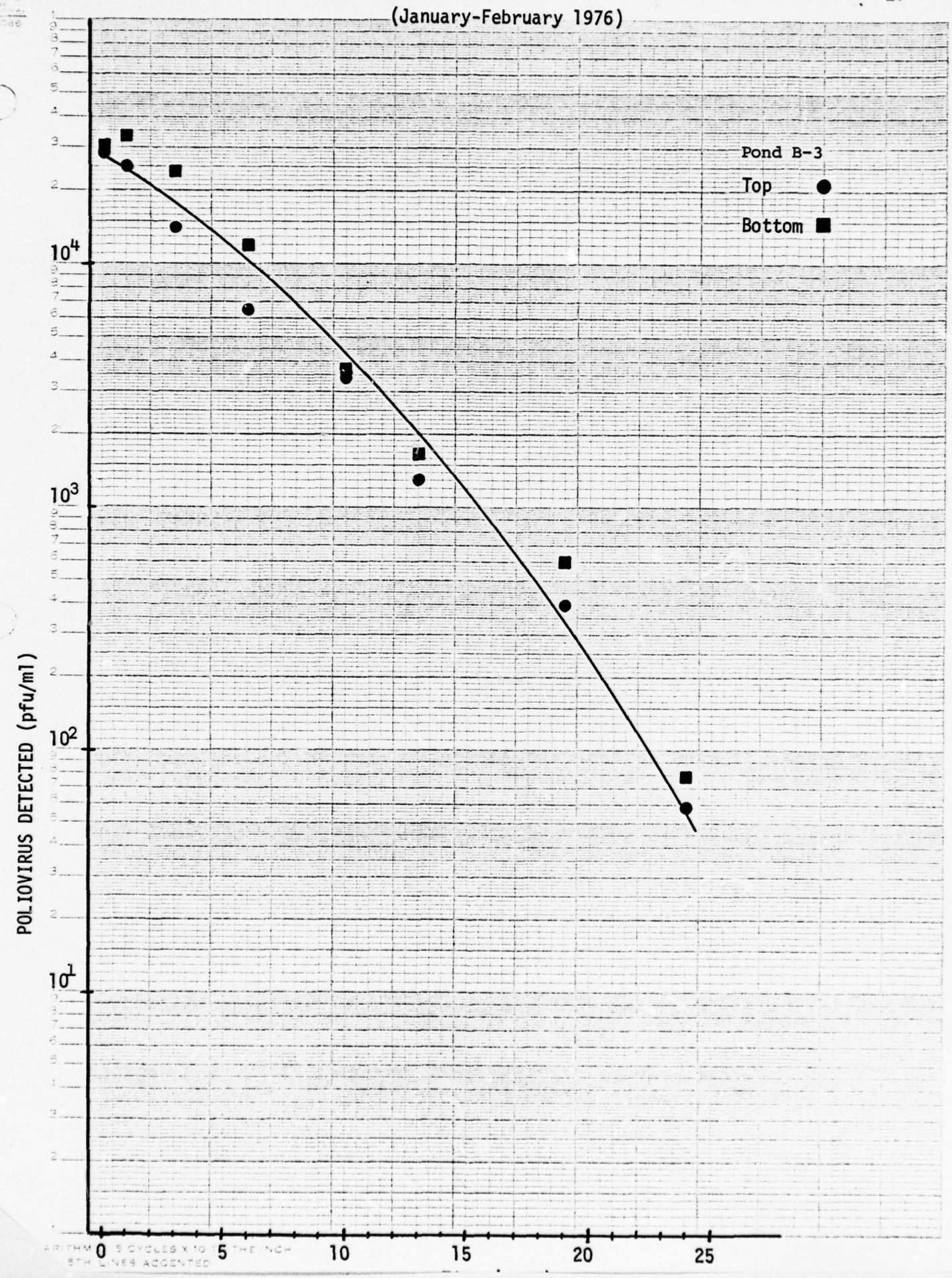


FIGURE 10. POLIOVIRUS SURVIVAL IN 42-INCH DEEP FINAL EFFLUENT POND
(January-February 1976)

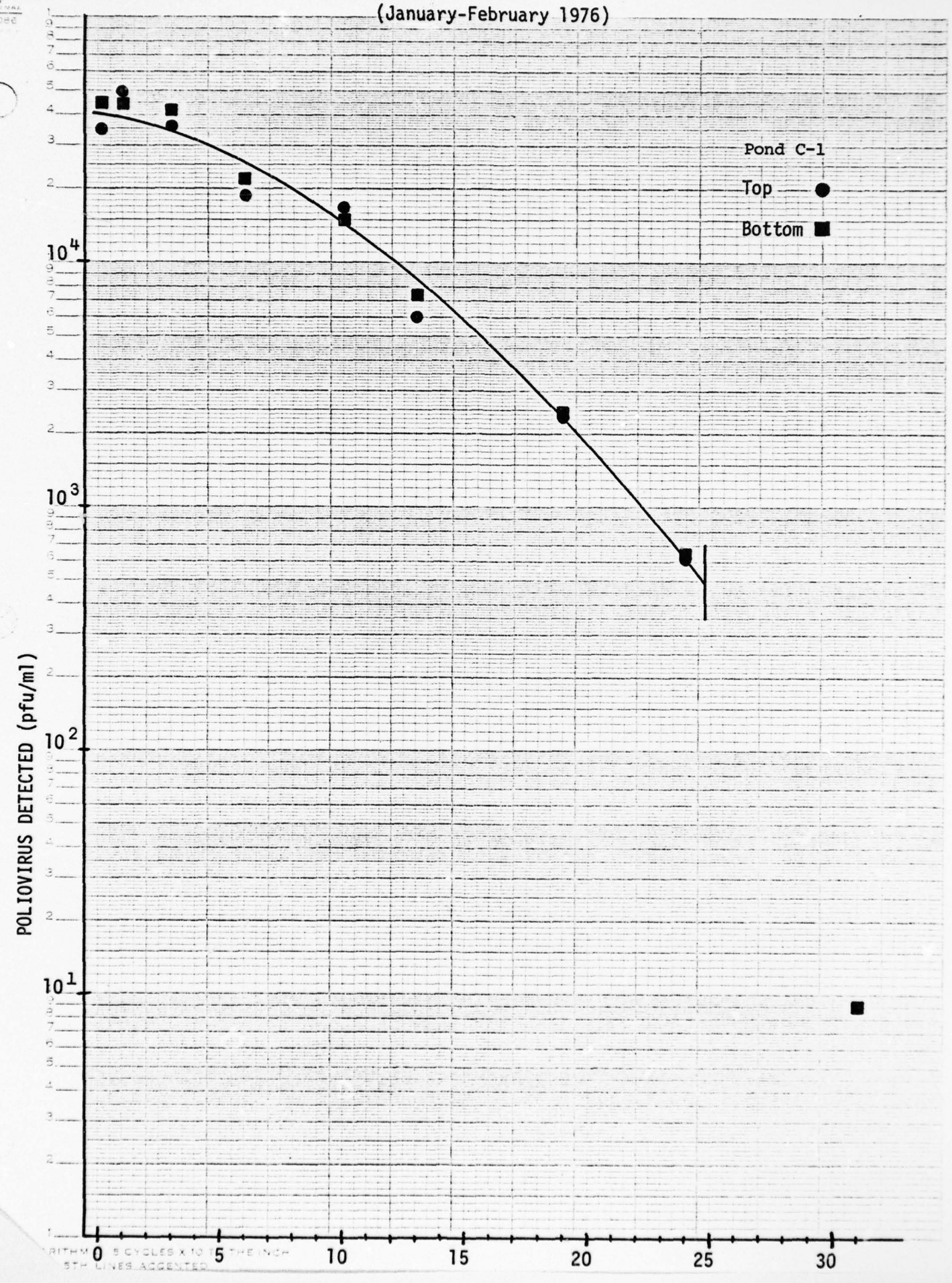


FIGURE 11. POLIOVIRUS SURVIVAL IN 90-INCH DEEP FINAL EFFLUENT POND
(January-February 1976)

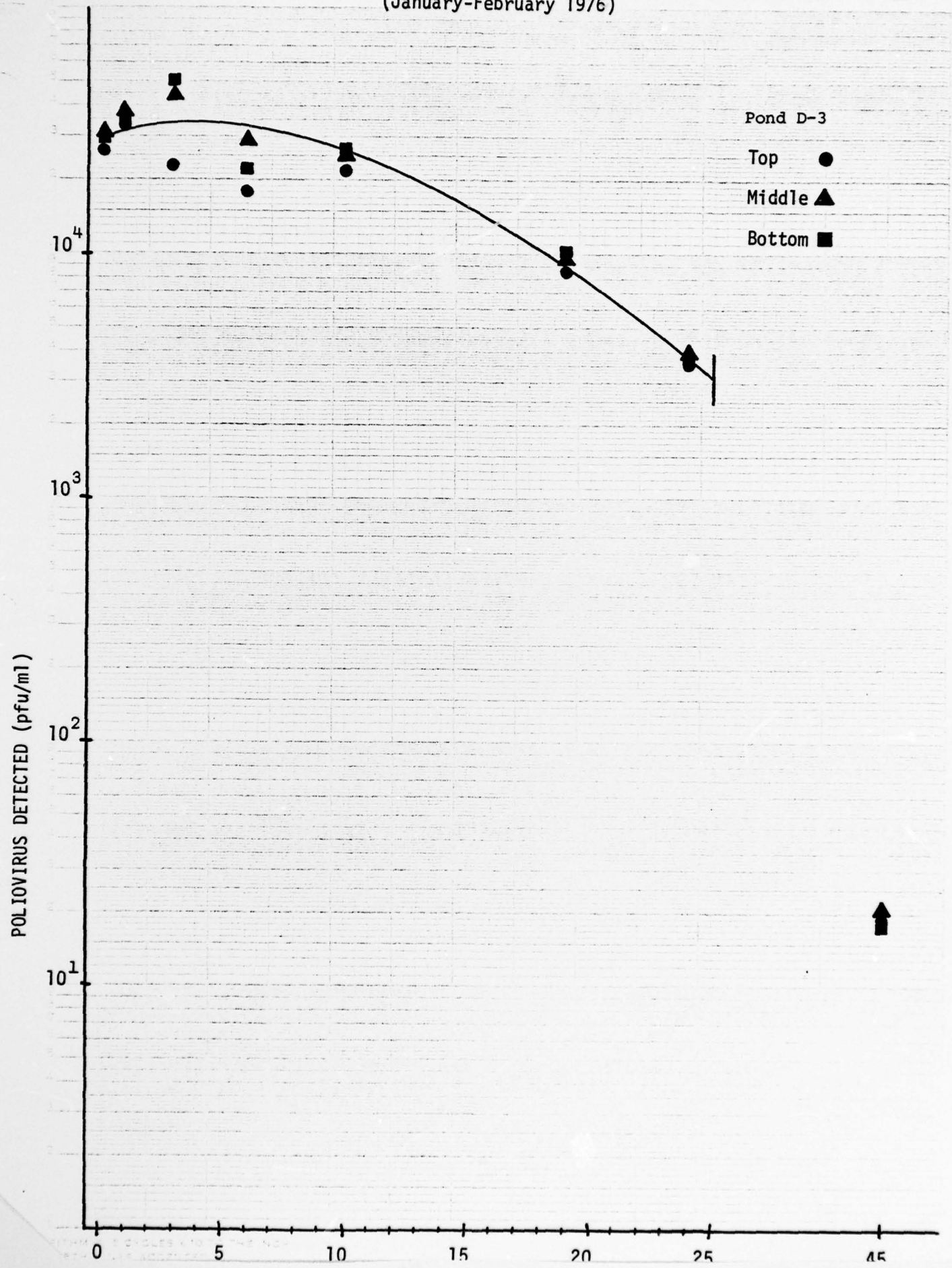


FIGURE 12. TEMPERATURE PROFILE - 30-INCH AND 90-INCH DEEP FINAL EFFLUENT PONDS
(March 31 - April 16, 1976)

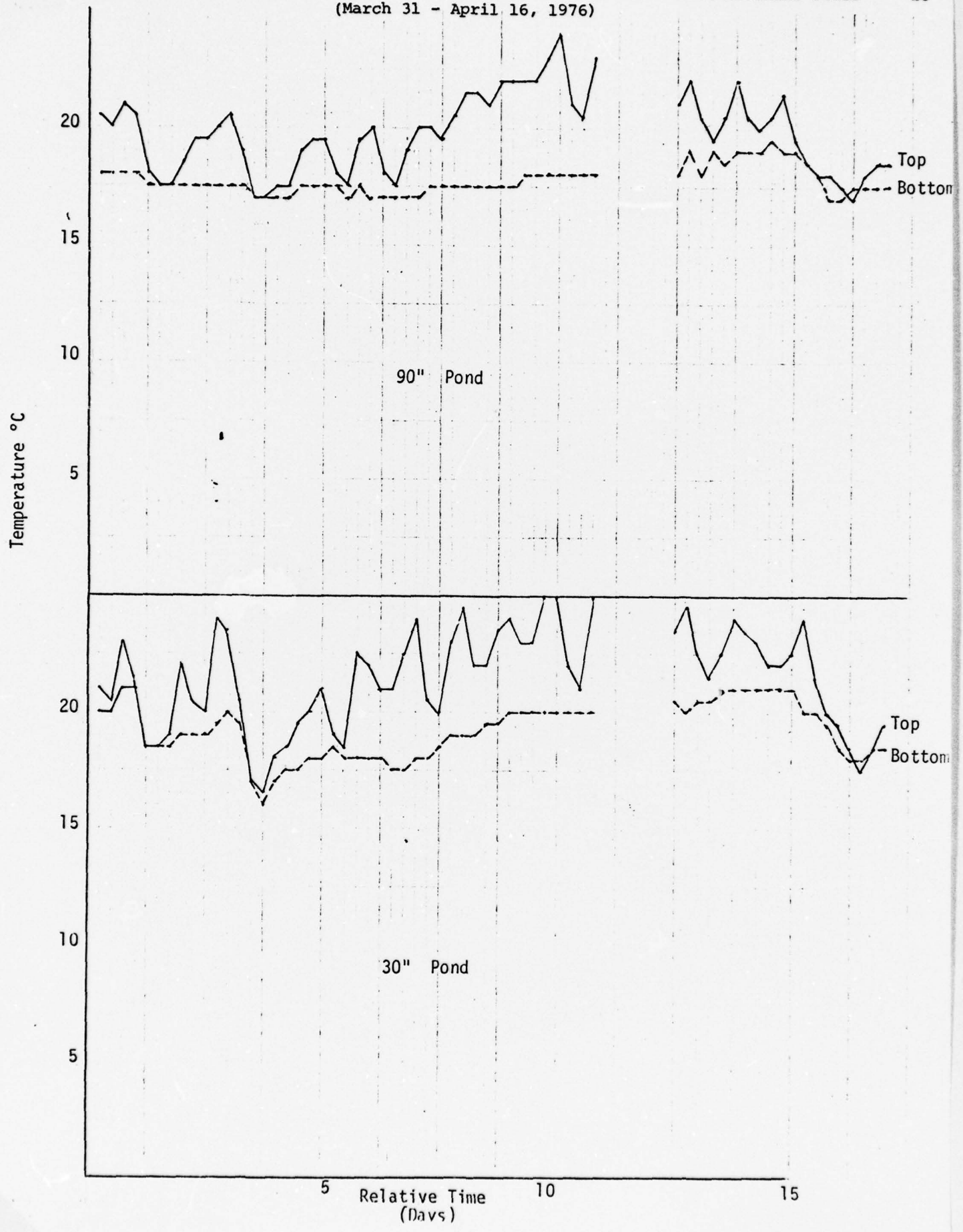


FIGURE 13. POLIOVIRUS SURVIVAL IN 30-INCH DEEP FINAL EFFLUENT POND
(March-May 1976)

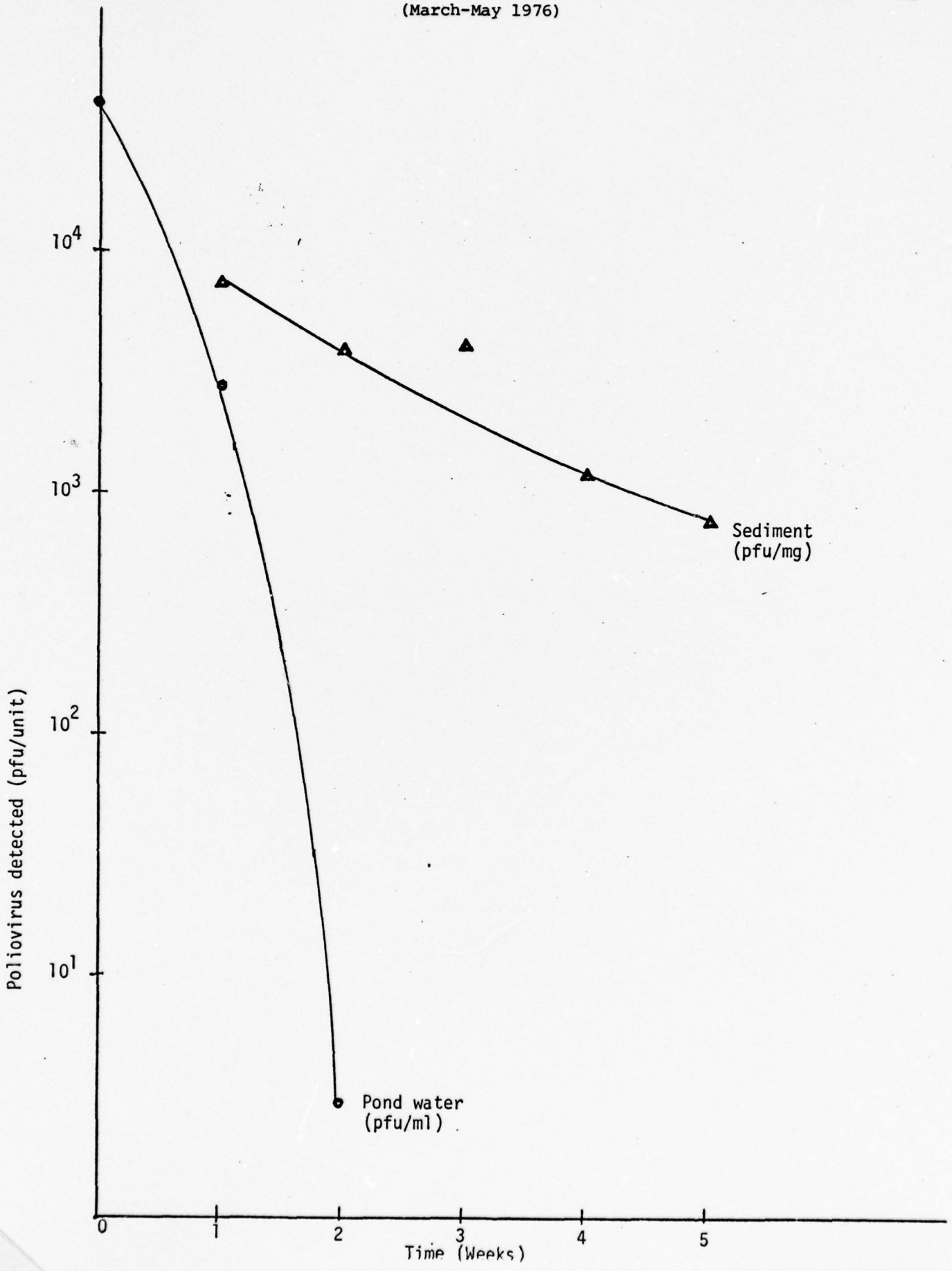


FIGURE 14. POLIOVIRUS SURVIVAL IN 90-INCH DEEP FINAL EFFLUENT POND
(March-May 1976)

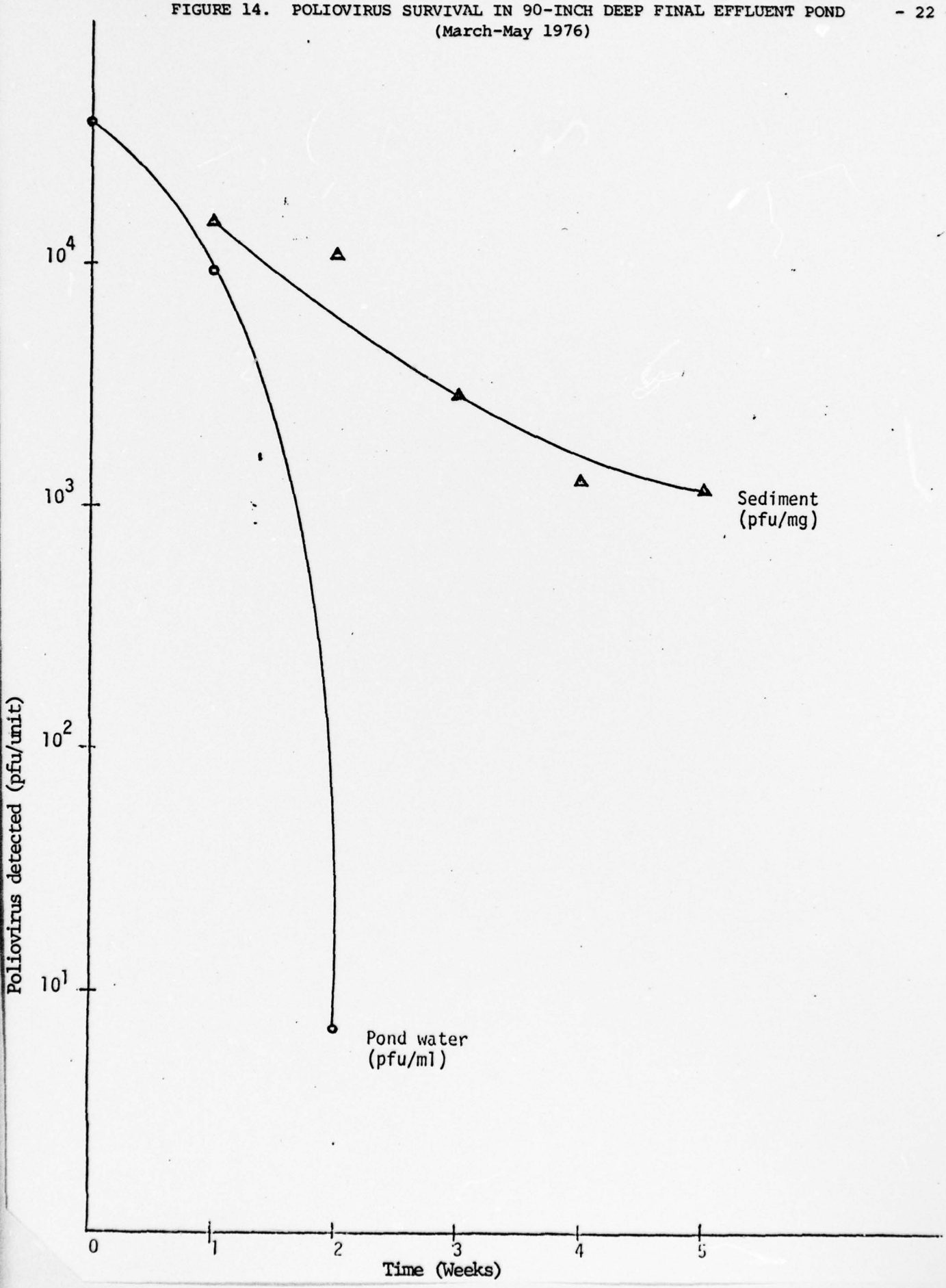


FIGURE 15. POLIOVIRUS SURVIVAL IN 30-INCH PRIMARY EFFLUENT POND
(March-May 1976)

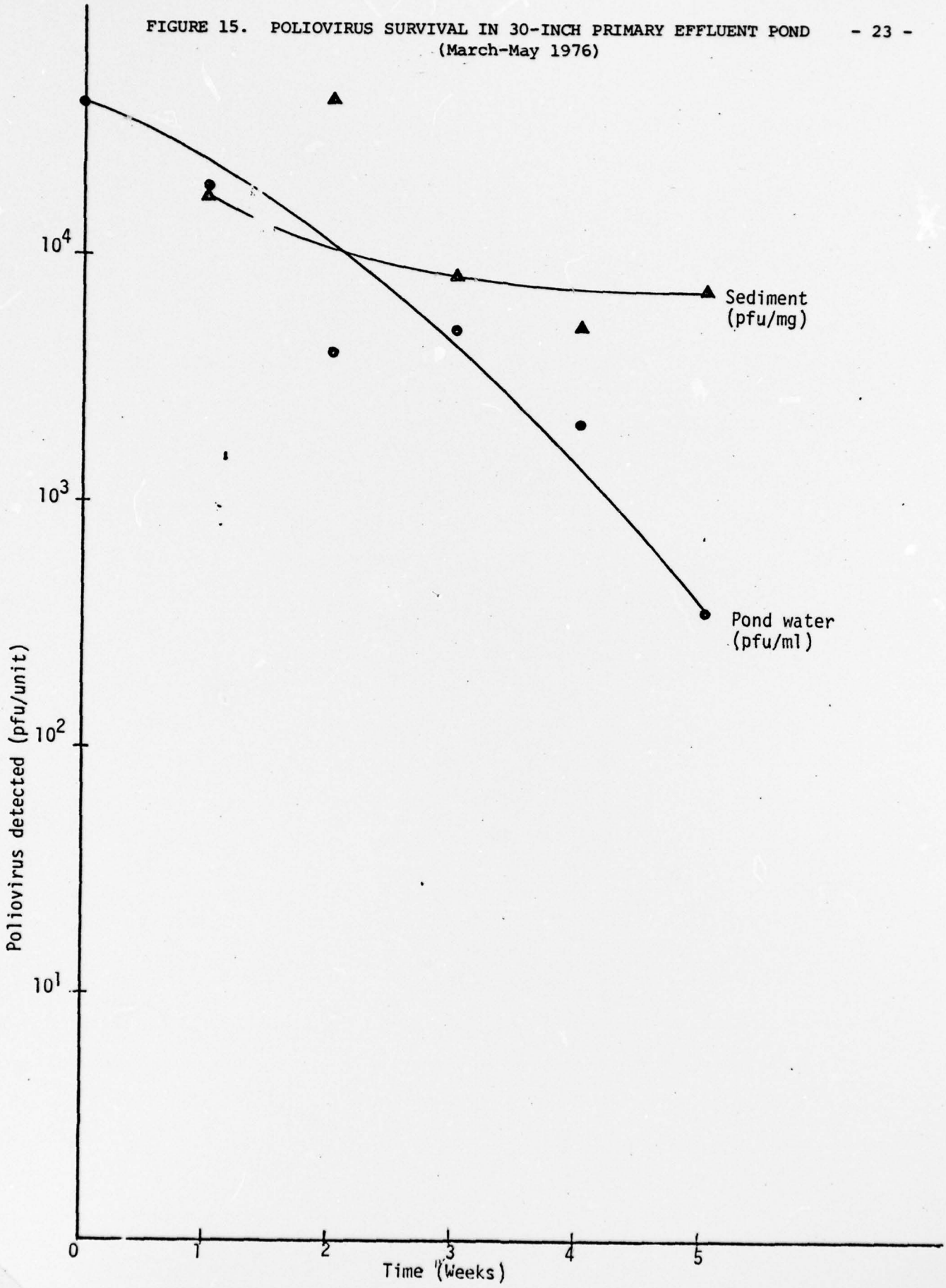


FIGURE 16. POLIOVIRUS SURVIVAL IN 90-INCH DEEP PRIMARY EFFLUENT POND
(March-May 1976)

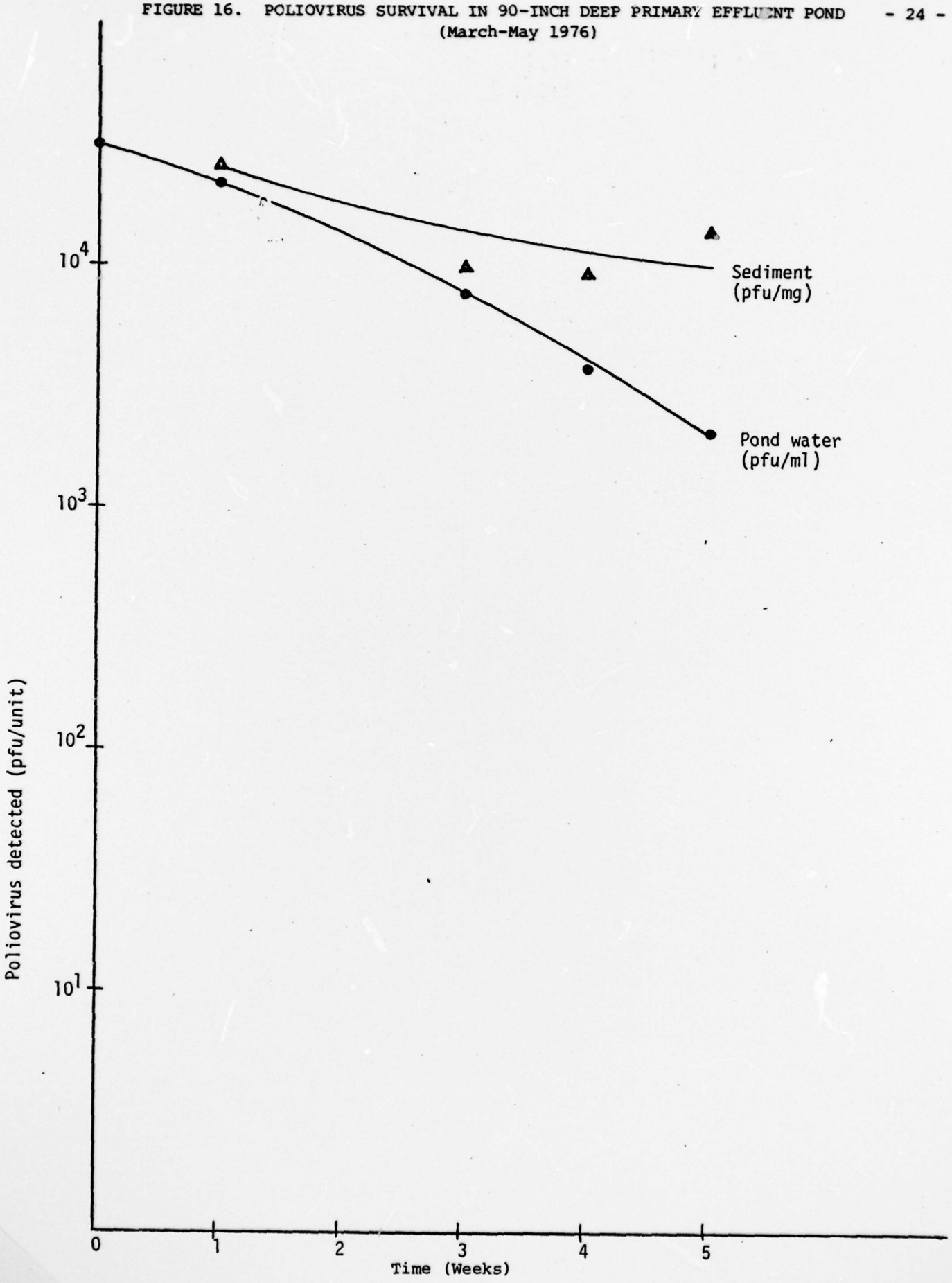


TABLE 1. Mean Temperature (September-October 1975)

Pond	Depth	Temperature (C)
A-1 (18")	Top	19
	Bottom	19
B-2 (30")	Top	19
	Bottom	18
C-1 (42")	Top	20
	Bottom	18
D-1 (90")	Top	20
	Middle	21
	Bottom	21

TABLE 2. CHEMICAL ANALYSES--POND B-3 (30-INCH)

Time (Days)	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	NO_3+NO_2 (mg/l) T	NO_3+NO_2 (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	Phosphates (mg/l) Total T	Phosphates (mg/l) Total B	Phosphates (mg/l) Ortho T	Phosphates (mg/l) Ortho B
1	35.3/10.3	53.7/15.0	24	23										
6	16.2/6.3	30.3/4.7	19	19	57	48	.21	.21	10.3	10.4	4.1	4.1		
13			17	18	41	43	.15	.15	10.6	10.6	3.4	3.2		
19	33.5/25/1	40.9/30.9	29	27	68	66	.16	.18	12.5	11.8			2.5	2.5
24	53.9/44.0	51.0/40.5	30	31										
31	68.8/61.3	24.1/17.6			194	86	.13	.10					2.2	2.5

TABLE 3. CHEMICAL ANALYSIS--POND D-3 (90-INCH)

Time (Days)	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	NO_2+NO_3 (mg/l) T	NO_2+NO_3 (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	Phosphates (mg/l) Total T	Phosphates (mg/l) Total B	Phosphates (mg/l) Ortho T	Phosphates (mg/l) Ortho B
1	6.4/5.2	16.2/8.2	28	27										
6	11.7/6.1	7.9/3.2	22	20	64	54	.17	.17	11.8	11.3	2.9	2.9		
13			16	19	39	39	.11	.10	10.6	10.8	2.7	2.8		
19		5.9/3.6	19	16	33	37	.09	.09	19.3	18.1			2.5	2.5
24	6.0/3.1	4.2/2.8	18	17										
31	109/94.6	5.7/4.5			210	47	.08	.08					2.3	2.4
38			34	26									3.3	4.6
45	74.6/68.4	14.7/12.2	57	26	216	115							2.6	2.8

TABLE 4. Poliovirus Isolated in Pond Sediments

Pond Number	Sampling Time (days)	Virus Eluted From Sediment (pfu/gm)
A-3	67	1
B-3	67	41
C-3	67	3
D-3	60	50

TABLE 5. CHEMICAL ANALYSIS--POND B-1 (30-INCH)

Time (Days)	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	NO_3+NO_2 (mg/l) T	NO_3+NO_2 (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	Phosphates (mg/l) Total T	Phosphates (mg/l) Total B	Phosphates (mg/l) Ortho T	Phosphates (mg/l) Ortho B
1hour	128.0/59.8	137.0/57.5	100	95	321	206	.083	.083	25.3	25.3	10.4	9.6	10.2	10.0
3	54.6/39.4	53.6/37.3	75	85	223	232	.086	.091	31.2	30.4	9.0	9.4	10.2	10.2
6	37.5/26.2	45.0/27.8	75	70	175	198	.086	.079	22.5	23.2	9.0	9.0	9.2	9.8
15	46.0/36.4	28.7/24.2	100	135	173	140	.1	.12	8.5	22.0	.6.8	9.6	6.8	9.7
22	54.4/42.0	52.0/43.2	46	47	105	109	.047	.047	7.1	6.9	7.8	7.4	7.0	7.0
29	25.4/22.6	30.6/21.0	35	40	135	133	.073	.082	6.5	6.5	7.5	7.7	7.2	7.1
34	119.2/112.4	16.5/12.2	86	43	286	94	.093	.07			8.5	7.3	7.5	7.5
43	27.6/25.4	25.0/20.4	48	48	96	95	.079	.1			5.7	7.3	5.5	6.8
50	151.6/139.6	33.4/22.8	82	35	263	113	.15	.08			6.3	6.8	4.7	6.7

TABLE 6. CHEMICAL ANALYSIS--POND D-1 (90-INCH)

Time (Days)	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	NO_3+NO_2 (mg/l) T	NO_3+NO_2 (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	Phosphates (mg/l) Total T	Phosphates (mg/l) Total B	Phosphates (mg/l) Ortho T	Phosphates (mg/l) Ortho B
1hour	138.0/86.4	165.4/109.0	110	95	242	296	.088	.083	28	31.3	10.4	9.6	9.3	9.7
3	47.0/38.0	46.2/38.5	95	95	209	216	.082	.097	35.8	34.2	8.4	8.4	8.8	8.6
6	32.6/24.4	39.2/24.4	85	90	198	240	.076	.11	26.0	30.2	8.4	9.0	9.2	8.7
15	37.3/31.4	30.2/25.2	80	85	164	198	.11	.16	22.7	28.4	7.4	8.0	8.1	9.0
22	77.2/60.0	63.6/48.0	59	47	151	125	.076	.7	30.4	29.2	9.6	8.0	8.5	8.2
29	54.0/44.0	61.8/27.2	43	72	149	252	.073	.15	18.5	31.5	7.3	10.2	5.8	10.1
34	104.4/95.1	145.0/134.5	76	49	208	131	.15	.133			8.5	7.8	7.5	7.5
43	70.8/56.8	47.6/40.0	51	58	113	128	.1	.175			6.8	8.7	6.0	8.5
50	41.6/28.0	81.0/60.4	31	62	100	203	.089	.22			6.8	11.6	6.7	10.2

TABLE 7. CHEMICAL ANALYSIS--POND B-3 (30-INCH)

Time (Days)	TSS/VSS (mg/l)		TOC (mg/l)		COD (mg/l)		NO_3+NO_2 (mg/l)		TKN (mg/l)		Phosphates (mg/l) Ortho	
	Top	Bottom	T	B	T	B	T	B	T	B	T	B
1 hour	81.0/29.3	153.8/32.3			73	78	.11	.16	14.8	15.9	6.7	6.7
3	30.2/17.5	41.9/17.5										
6	57.5/40.9	20.9/15.0	34	30	120	81	.12	.10	7.7	11.2	5.0	7.0
15	73.4/53.0	68.4/43.2	56	61	138	118	.29	.14	3.4	5.5	1.5	4.2
22	80.8/63.6	75.0/61.0			98	100	.09	.09	2.2	2.7	1.7	2.4

TABLE 8. CHEMICAL ANALYSIS--POND D-3 (90-INCH)

Time (Days)	TSS/VSS (mg/l)		TOC (mg/l)		COD (mg/l)		NO_3+NO_2 (mg/l)		TKN (mg/l)		Phosphates (mg/l) Ortho	
	Top	Bottom	T	B	T	B	T	B	T	B	T	B
1 hour	19.3/10.2	20.0/10.2			62	65	.16	.16	18.1	18.1	6.0	6.1
3	5.4/2.4	13.3/7.9										
6			21	27								
15	50.2/39.6	15.5/10.8	63	32	151	67	.20	.28	5.7	13.5	2.3	5.5
22	51.4/48.6	58.0/43.0			62	62	.7	.72		7.1	3.7	3.8

TABLE 9. SEDIMENT ANALYSIS FINAL EFFLUENT PONDS[a]

30-Inch Pond

Time (weeks)	Weekly Sediment Deposition (mg)	pfu/mg weekly	pfu/mg cumulative
1	900	7.1×10^3	--
2	580	5.2×10^2	3.8×10^3
3	620	7.4×10^1	4.1×10^3
4	371	--	1.2×10^3
5	388	--	7.9×10^2

90-Inch Pond

1	800	1.5×10^4	--
2	400	3.3×10^2	1.1×10^4
3	700	9.7×10^2	2.9×10^3
4	507	5.9×10^1	1.3×10^3
5	515	5.8×10^1	--

[a] Values reflect results obtained per sampler. The volume of each sampler represents 1% of the total bottom area of the pond.

TABLE 10. SEDIMENT ANALYSIS PRIMARY EFFLUENT PONDS[a]

30-Inch Pond

Time (weeks)	Weekly Sediment Deposition (mg)	pfu/mg weekly	pfu/mg cumulative
1	780	1.9×10^4	--
2	600	2.3×10^4	4.3×10^4
3	1160	1.2×10^3	8.4×10^3
4	1042	1.1×10^2	5.2×10^3
5	660	1.2×10^2	7.2×10^3

90-Inch Pond

1	1200	2.7×10^4	--
2	440	4.4×10^4	3.9×10^4
3	240	1.2×10^4	1.0×10^4
4	564	5.3×10^3	9.7×10^3
5	1274	4.9×10^3	1.4×10^4

[a] Values reflect results obtained per sampler. The volume of each sampler represents 1% of the total bottom area of the pond.

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